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Synthetic Lethal Gene for PTEN as a Therapeutic Target

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14. ABSTRACT

PTEN is the gene which is down-regulated in the majority of prostate cancer, and PTEN-Akt pathway is considered to play a critical role in radiation resistance. The overall goal of this project is to identify a specific gene that is essential for this important pathway in the hope that targeting such gene will sensitize PTEN-negative tumor stem cells to be radiation-sensitive. To accomplish this goal, we will isolate cancer stem cells from patients and prepare PTEN-deficient cells. We will then screen genes that play critical roles in the PTEN pathway using a technique called shRNA library screening, with or without radiation treatment of these cells. When we identify a gene, we will then test the effect of such gene in an animal model to test our hypothesis. We believe that identifying such genes will lead to development of a novel therapeutic approach to radiation-resistant prostate cancer.

15. SUBJECT TERMS

Prostate cancer, tumor stem cells, PTEN, resistance

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INTRODUCTION

Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer death among men in the US. Down-regulation of tumor suppressor gene, PTEN, has been found in up to 60% of advanced prostate cancer, and therefore, PTEN is considered to play a critical role in tumor progression of prostate cancer (1-3). PTEN is a phosphatase that antagonizes the phosphoinositol-3-kinase/AKT signaling pathway and suppresses cell survival as well as cell proliferation. PTEN is also known to suppress self-renewal of CSC which is believed to be responsible for chemo-resistance. Therefore, down-regulation of PTEN and concomitant activation of AKT pathway endows tumor cells with survival advantage during chemo- and radiation-therapy (4). We hypothesize that there are synthetic lethal genes that are up-regulated when PTEN function is lost in prostate cancer. Knock-down of such gene in PTEN-negative CSC is expected to be lethal when they are treated with radiation. The purpose of this project is to identify such gene(s) by particularly focusing on kinase genes. We expect that such gene will be an excellent therapeutic target to overcome resistance to chemo-, radiation and hormone-therapy for prostate cancer patients. The main objective of this project is to identify synthetic lethal gene(s) in PTEN-negative prostate CSC.

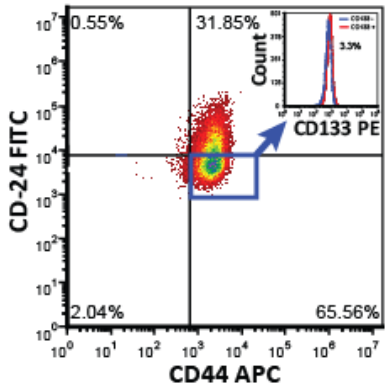
BODY

The main objective of this project is to identify synthetic lethal gene(s) in PTEN-negative prostate CSC. To test our hypothesis, we will first isolate CSC from patients and prepare PTEN-knockdown cells (**Aim 1**). We will then a screen shRNA library for synthetic lethal genes in these cells with or without radiation treatment (**Aim 2**). When we identify a synthetic lethal gene, we will then test the effect of such gene in an animal model (**Aim 3**).

Aim 1. To isolate CSC from patients and prepare PTEN-knockdown cells

Progress

We have isolated CSCs population using CD24-/CD44+/CD133+ from various prostate cancer cell lines including PC3mm and C4-2B. Our initial FACS analysis indicates that PC3mm has around 3% of stem cell population (Fig. 1A). When they are cultured in serum-free medium, they generated significantly higher numbers of large prostatspheres. We also transplanted the CSCs prepared from PC3mm into nude mice and found that they have significantly stronger abilities to initiate tumorigenesis compared to the original cells as shown in Fig. 1B.



# of Cells	10^4	10^3	10^2
Stem ($CD24^-/CD44^+/CD133^+$)	5/6	3/6	1/6
Non-Stem	2/6	1/6	0/6

Preparation of cancer stem cells. (A) Tumor stem population in PC3mm was analyzed by FACS using specific antibodies to CD24, CD44, and CD133. (B) Tumor stem cells isolated from PC3mm were injected subcutaneously into nude mice, and the growth of tumor was monitored by Xenogen bioimaging system.

We have obtained the similar results for CSCs from C4-2B cells. We have also obtained shRNA expressing lentiviral vector (SBI-Bioscience) against the PTEN gene. We have already prepared high-titer lentiviruses and infected them to PC3mm and C2-4B cell lines followed by establishing cell lines by puromycine screening. Therefore, these materials will be used in Specific aim 2.

We are trying to isolate CSCs from clinical samples, and we made an arrangement to obtain samples from Tissue Repository Core at our Cancer Institute. We plan to isolate CSCs by first eliminating non-tumor cells using lineage marker antibodies followed by soring CSCs using the ALDH marker by FACS. The isolated CSCs will be examined for their tumor initiating abilities using nude mice. Therefore, Aim 1 is in progress.

Aim 2. To screen shRNA library for synthetic lethal genes in these cells with or without radiation treatment.

Progress

We have already obtained multiple shRNA libraries from Addgene. These include the DECIPHER Lentiviral shRNA Library with barcodes and they are targeted to pathway, disease, and cell surface, respectively. We have prepared these lentiviruses and tested for the titers of each preparation. We are ready for infection to CSCs. On the other hand, we have also established cell lines that express shRNA libraries by screening puromycine-resistant cells. Therefore, we can also start our screening using these cell lines, and these tools provide us with some flexibility. On the other hand, we are currently testing the best condition of radiation for prostate CSCs. Our dose is 0.25, 0.5, 1.0 and 2.0 Gy. We hope we will find out the sub-lethal conditions for each cells soon.
Therefore, the experiment for Aim 2 is in progress.

Aim 3. When we identify a synthetic lethal gene, we will then test the effect of such gene in an animal model

Progress

The experiments for Aim 3 are dependent on the identification of specific gene in Aim 2. Therefore, we are not currently conducting these experiments.

KEY RESEARCH ACCOMPLISHMENTS

1. We were able to isolate CSCs from multiple prostate cancer cell lines and validated their tumor initiating abilities *in vivo*.
2. We have established PTEN knockdown cell line of prostate cancer.
3. We have successfully prepared lentiviruses of three shRNA libraries.
4. We have established PC3mm cell line that has shRNA library for screening.

REPORTABLE OUTCOMES

Peer reviewed publications
None.

Employment
1. Ms. Ying Liu (Graduate student) has been partly supported by the current grant.

CONCLUSIONS

This project has been delayed due to the relocation of our entire lab to University of Mississippi Medical Center, and we needed to re-establish our lab setting including personnel. However, we have already established basic material and tools for the shRNA library screening. Because this is a discovery project and needs screening of the libraries, we have not had scientifically reportable outcome yet. However, we expect that the screening will be done within the next 3-4 moths.

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